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DNA Isolation V.1

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

2023 NUS-Singapore iGEM team followed this protocol to isolate the DNA fragments from the agarose gel after the gel electrophoresis.

Protocol materials

Buffer QG **Qiagen Catalog #**19063

Buffer PE **Qiagen Catalog #**19065

Safety warnings

- Proper lab PPE must be worn at all times.
- When using the LED transilluminator, wear eyewear that is designed to block blue light to protect the eyes.

1 Prepare and label an Eppondoft tube.

2 Place the agarose gel onto the LED Transilluminator (blue light) to observe the DNA band(s).

Safety information

- Wear protective eyewear to protect the eyes from blue light.
- Turn off the LED transilluminator immediately when it is not in use.

3 Cut out the target DNA band from the agarose gel.

4 Put the gel piece into the Eppendorf tube.

5 Add 450 µL of Buffer QG **Qiagen Catalog #**19063 into the Eppendorf tube.

6 Heat the Eppendorf tube at 55 °C for 00:20:00 in the Thermo-Shaker. 20m

7 Add 150 µL of 100% isopropanol (IPA) into the Eppendorf tube and shake the tube to mix the solution well.

8 Transfer the whole solution into a QIAquick Spin Column (purple tube with a maximum volume of 750 µL).

9 Centrifuge it for 13 rpm, 00:01:00 . 1m

10 Discard the flow-through and place the QIAquick column back into the same tube.

11 Add 700 µL of Buffer PE **Qiagen Catalog #**19065 into the QIAquick column.

12 Centrifuge it for 13 rpm, 00:01:00 .

13 Discard the flow-through and place the QIAquick column back into the same tube.

14 Add 700 µL of Buffer PE **Qiagen Catalog #**19065 again into the QIAquick column.

15 Centrifuge it for 13 rpm, 00:01:00 .

16 Discard the flow-through and place the QIAquick column back into the same tube.

17 Centrifuge the emptied QIAquick column at 13 rpm, 00:01:00 to remove residual Buffer PE **Qiagen Catalog #**19065 . 1m

18 Transfer the QIAquick column into the newly labelled Eppendorf tube.

19 Add 30 µL of DI water into the QIAquick column.

20 Centrifuge the tube at 13 rpm, 00:01:00 , ensuring that the direction of the Eppendorf tube's cap is the same as the direction of spinning to avoid breaking. 1m

21 Discard the QIAquick column, the solution left in the Eppendorf tube contains the DNA fragment of interest.

22 Use the Nanodrop to measure and record the purity and concentration of the DNA fragment.

Equipment

NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer	NAME
UV-Vis Spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU

23 Keep the isolated DNA fragment in the Room temperature .